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Optimization of extraction conditions in the air-conditioned Macfadyen funnel extractor for soil arthropods

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With 3 figures

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1. Introduction

Edwards & Fletcher (1971) considered the Macfadyen air-conditioned funnel extractor (Macfadyen 1961) as the most efficient device for extracting soil microarthropods. Since then, three modified Macfadyen extractors were constructed in Switzerland (Bieri *et al.* 1978a) following a prototype at the Rothamsted Experimental Station (UK). In each unit, 96 soil samples can be extracted simultaneously. Strictest consideration was given to an environmentally independent regulation system of the extraction conditions. This enables temperature variations at the upper surface of the soil samples of between 15 °C and 70 °C or the maintenance of a preselected temperature (± 1 °C) during the extraction. Furthermore, the humidity of the air above samples can be regulated to some extent so that desiccation of the samples proceeds slowly from the top, thus influencing the migration of the microarthropods. On the other hand, the lower surfaces of the soil samples are cooled by circulating cold moistened air vented by a 3/4 hp (600 W) compressor. In preliminary tests (Table 1) it was established that temperature differences between the upper and lower surfaces of the soil samples were greatest when the temperature at the upper surface was high.

The preliminary results obtained with the new extractors were quite satisfactory (Bieri *et al.* 1978a). However, the temperature gradients in samples were established empirically. Experiments were therefore made to establish the real potential of the extractors in connection with different soil types and, in addition, attempts were made to minimize handling and counting time per soil sample.

At first it was necessary to clarify the effect of constant light and to test the effect of light quality on microarthropod activity. Secondly, it was necessary to consider the effects of variation in the thickness of the sample in the extraction cylinder of intact or crumbled soil. Finally, extraction conditions were optimized considering the most convenient

Table 1. Temperature gradients in crumbled soil samples in relation to sample thickness (Th) and soil type (sandy loam: SL; bog soil: BS)

Temperature at sample top	Gradients in °C			
	Th = 2 cm		Th = 4 cm	
	SL	BS	SL	BS
30 °C	3	2	5	5
35 °C	4	6	6	8
40 °C	—	7	10	13

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light and sample thickness, and the number of extracted organisms was compared with that obtained by the flotation method (BIERI & DELUCCHI 1980). A technique to rapidly separate extracted organisms from soil particles was refined.

2. Material and methods

2.1. The effect of intensity and quality of light on the extraction efficiency

The cover of the extractor containing incandescent and fluorescent lamps (BIERI *et al.* 1978a) was divided in two equal sections A and B by a plate of polystyrene foam protected with aluminium foil. In section A the fluorescent lamps were removed, so that the light of visible red and the infrared predominated. In section B both lamp types were maintained.

In a sandy forest soil along the Aare river near Brugg (Canton Aargau) 24 samples (ca. 266 cm³ each) were taken from a small homogeneous area using a special soil corer (BIERI *et al.* 1978b). The samples were crumbled and half of them were placed in section A and the other in section B. The final size of each sample in the extraction cylinder was 6 cm thick and 9.5 cm in diameter and soil organisms were extracted for 7 days. The temperature gradient in the samples was 6 °C with a temperature at the upper surface of 29 °C. The soil organisms were collected in glass vials containing isopropanol.

2.2. The effect of thickness and texture (intact or crumbled) of the soil sample on extraction efficiency

To define the most appropriate thickness and state of preparation of the soil samples for maximum extraction of the most common categories of soil arthropods, 48 cores (ca. 266 cm³ each) were taken from a small homogeneous area of a brown gley soil near Bülach (Canton Zurich).

The soil cores were extracted for 150 h as follows:

- 12 samples were left in their plastic tubes (inserted in the soil corer before the sample was taken and used for transportation from the field; see BIERI *et al.* 1978b) and placed upside down on the sieves of the extraction cylinders. The space between cylinder wall and plastic tube was filled with polystyrene foam.
- 36 samples were crumbled separately; 12 of them were placed in unmodified extraction cylinders giving a final soil mass for each cylinder of ca. 4 cm thickness, a second and third series of 12 soil samples were placed in cylinders with smaller diameters of 6.8 and 9.5 cm, respectively. In the cylinders with the smallest diameter the final soil mass had a thickness of 11 to 13 cm, whereas in the cylinders of 9.5 cm the thickness was 6 to 7 cm.

The following temperatures were recorded above and below samples:

- sample intact, 10 cm thick, top 33 °C, bottom 23 °C;
- sample crumbled, 11—13 cm thick, top 33 °C, bottom 23 °C;
- sample crumbled, 6—7 cm thick, top 30.5 °C, bottom 23 °C;
- sample crumbled, 5 cm thick, top 29.5 °C, bottom 23 °C.

The numbers of extracted microarthropods were analyzed by analysis of variance using the non-parametric method of KRUSKAL & WALLIS (1952, 1953) implemented in the SPSS standard software package of NIE *et al.* (1975).

2.3. Estimation of optimum temperature gradients in combination with sample thickness of two soil types for maximum extraction

Samples of a typical mineral soil and of a soil of high organic content were taken from fodder cornfields at the Agricultural School of Inst (Canton Bern). Both soils had the same climatic conditions, the same crop, and had been cultivated in the same manner. The mineral soil was a calcareous sandy loam originating from a moraine of the last glacial epoch, while the bog soil contained mainly decayed peat moss from an old shallow bog.

To obtain the necessary information on the extraction conditions inside a soil sample the temperatures were periodically measured at 3 levels (above, middle, below) of the sample using copper constantan bimetal sensors. Desiccation patterns were observed on additional cores placed in the Macfadyen extractor and analyzed at regular intervals to determine the loss of water at 3 levels ($^1/_3$ top, $^1/_3$ middle and $^1/_3$ bottom) of the samples by drying them at 105 °C for 24 h.

The temperature gradient and the temperature at sample top in the extraction cylinder are given in table 1.

For each temperature gradient to be examined 48 samples (of ca. 266 cm³) were taken. Twelve of them were immediately deep frozen (—20 °C) and extracted later by flotation (BIERI & DELUCCHI 1980); 36 samples were carefully crumbled and processed by the Macfadyen extractor with 2 different thicknesses, i.e. 4 and 2 cm. One third of these samples was used to study the desiccation patterns.

The thickness of 2 cm was considered in this experiment as a follow up of the results obtained under 2.2). To measure the efficiency of a temperature gradient, the extracted Collembola and Acari were compared with the number obtained by flotation. The duration of the extraction was 140 to 150 h.

Each temperature gradient was tested separately. For each gradient, however, the samples of sandy loam and bog soil were processed simultaneously; the different temperatures registered for the two soil types during the same experiment are due to their different physical characteristics.

The Collembola were identified to the family level, whereas the Acari were differentiated in "Gamasidae" and "other mites". The counts were analyzed as described above in Section 2.2.

2.4. The counting technique

The arthropods obtained from the Macfadyen extractor fell into vials containing isopropanol. It was almost impossible to obtain "clean" samples of animals, as small soil particles also fell into the vials during extraction. Up to 90% more time was needed to count animals in "dirty" samples as compared with "clean" samples.

To separate heavier soil particles from the animals, the content of a vial was poured into a tube (3 cm diam. × 6 cm) filled $^2/_3$ with glycerol. Soil particles sank to the bottom, while the animals floated at the interphase between the glycerol and isopropanol. After 2 or 3 min the isopropanol could be decanted together with the arthropods into a counting dish.

To get an estimate of the number of animals lost using the glycerol method 76 soil samples of 44 different sizes (133, 266, 362 and 724 cm^3) were taken from a greenhouse (where no pesticides had been applied) and processed in the Macfadyen extractor. The samples of each size (= 19) were split into 2 groups: the arthropods of 9 samples were sorted by hand, whereas the arthropods of the other 10 were counted after cleaning with glycerol. The counts were transformed to square roots and compared by Student's t-test.

3. Results

3.1. Effect of the intensity and quality of light on extraction efficiency

Only the most abundant taxonomic categories of microarthropods were considered for statistical analysis. The results indicated that light has no repellent effect on the micro-arthropod categories considered. However, mean numbers were generally slightly higher with illumination by fluorescent tubes and subsequent extractions were therefore made with fluorescent light.

3.2. The effect of thickness and texture of the soil sample on extraction efficiency

The most efficient extraction was obtained for all taxonomic categories considered when samples were crumbled and 4 cm thick in the extraction cylinder (Table 2). High numbers of mites were also extracted from the 10 cm thick intact soil samples; a similar result has been obtained by EDWARDS & FLETCHER (1971). Collembola seem to have some difficulties to leave thick samples, independently of their texture (intact or crumbled).

Table 2. Effect of thickness (in cm) and texture of soil samples of constant volume on extraction efficiency

Taxonomic categories	Mean number per Sample				Mean ranks			
	Intact	Crumbled			Intact	Crumbled		
	10 cm	11—13 cm	6—7 cm	4 cm	10 cm	11—13 cm	6—7 cm	4 cm
Collembola								
Sminthuridae	1.4	0.4	1.4	2.9	24.75	13.96	24.46	**34.83**
Poduridae	0.5	0.4	0.5	5.4	22.25	20.63	23.54	31.58
Onychiuridae	0.5	0.4	2.3	11.2	16.62	15.50	24.21	**41.67**
Isotomidae	0.9	0.4	2.2	16.8	19.21	13.67	26.08	**39.04**
Entomobryidae	0.4	0.5	0.8	1.3	22.33	23.00	24.33	28.33
Acari	46.4	16.6	34.8	52.4	28.35	10.96	24.29	**34.50**

Note: Bold mean rank numbers: significantly higher numbers of animals with a probabilityof 90% ($p \leq 0.01$).

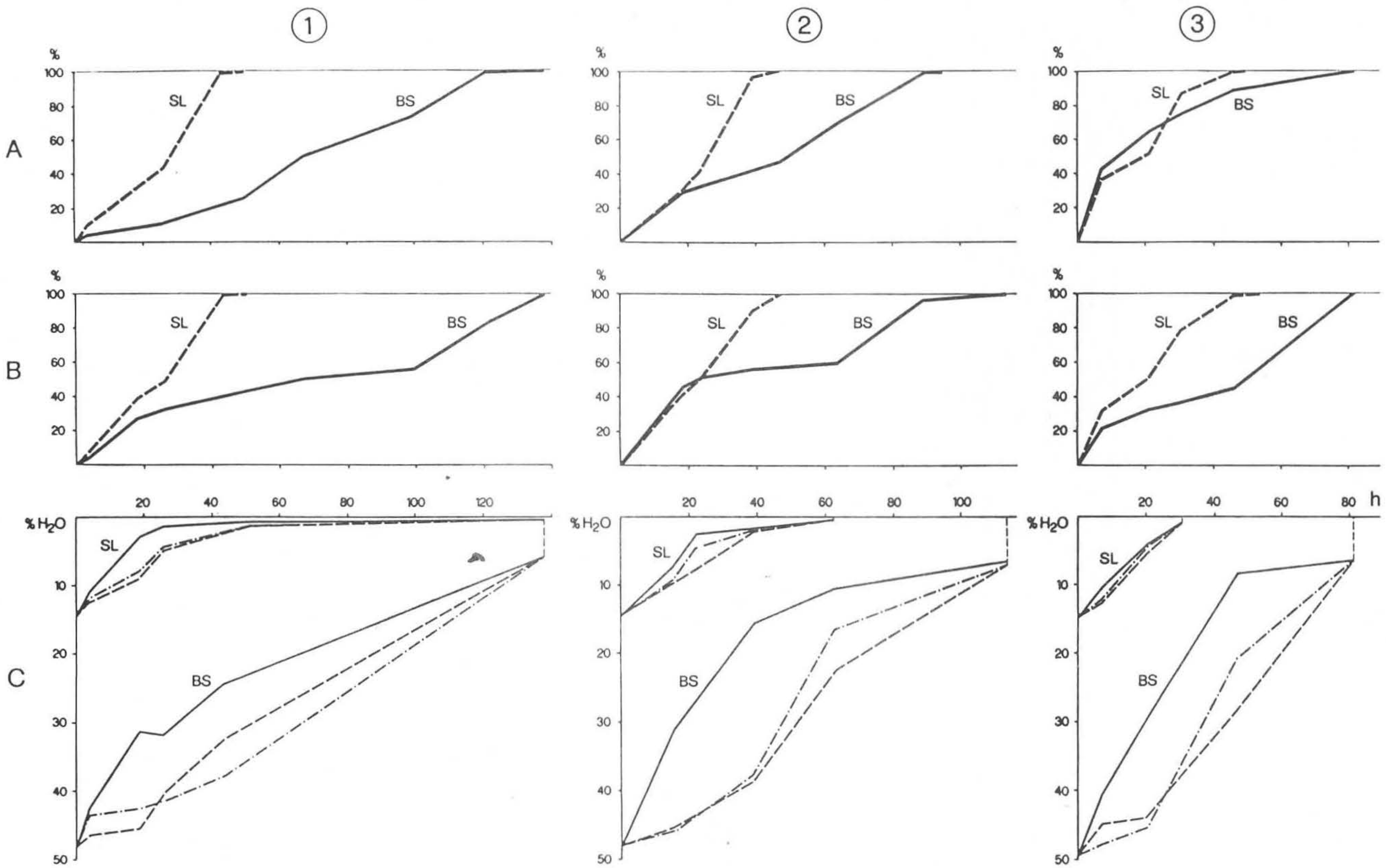

Fig. 1. Cumulated numbers of extracted Collembola (A) and Acari (B) from sandy loam (SL) and bog soil (BS) using crumbled samples of 4 cm thickness in a modified Macfadyen extractor. Temperature at sample top 30 °C: (1); 35 °C: (2) and 40 °C: (3). Water content of the sample (C) measured in the top (———), the middle (— · —) and bottom (— — —) of the sample (h = hours).

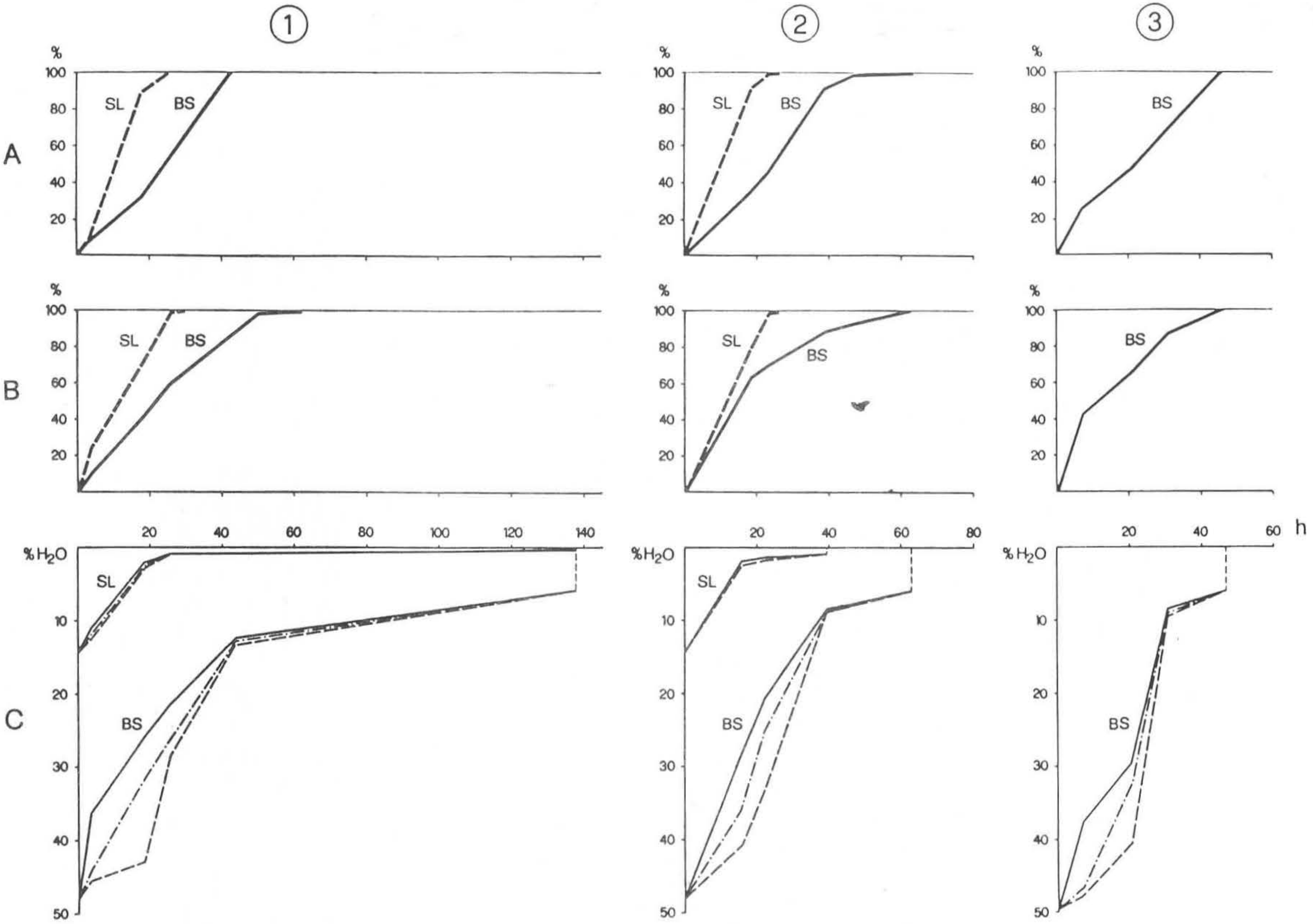

Fig. 2. Cumulated numbers of extracted Collembola (A) and Acari (B) from sandy loam (SL) and bog soil (BS) using crumbled samples of 2 cm thickness in a modified Macfadyen extractor. Temperature at sample top 30 °C: (1); 35 °C: (2) and 40 °C: (3). Water content of the sample (C) measured in the top (———), the middle (— · —) and bottom (— — —) of the sample (h = hours).

3.3. Estimation of optimum temperature gradients in combination with sample thickness

Figs. 1 and 2 report the water content of the soil samples at the top, middle and bottom together with the number of Collembola and Acari observed during the whole extraction period (up to 150 h). Irrespective of the temperature at the top of the 2 cm thick samples in the extraction cylinder, the temperature gradient accross the samples and desiccation time is about 50 % of those with 4 cm thick samples. However, as the gradient increases desiccation time decreases in both soil types.

The distribution of the extracted microarthropods over time has been followed by counting Collembola and Acari at intervals of 8 to 30 h. Only extractions performed simultaneously on samples taken at the same time and under identical conditions can be directly compared, otherwise comparison must be made on a relative basis. In general numbers of microarthropods were highest when samples were taken in July (temperature at sample top = 35 °C).

Nearly 100 % of Collembola and Acari are extracted in ca. 45 h from crumbled sandy loam samples of 4 cm thickness independent of the temperature gradient and of the temperature at sample top; the differences between the distribution curves (Fig. 1) are very small. With bog soil samples of the same thickness it takes longer to obtain nearly 100 % of Collembola and Acari: 120 to 150 h when the temperature at sample top is 30 °C, 90 to 110 h when temperature is 35 °C, and about 80 h when temperature is 40 °C. Compared to the flotation method (Fig. 1, Table 3) it appears that the Macfadyen extractor gives better results in the case of sandy soil provided that the temperature at sample top does not exceed 35 °C. In the case of bog soil, the Macfadyen extractor shows the same advantages against flotation for the Acari as in the case of a sandy loam, whereas the situation is reversed for the Collembola.

If the thickness of crumbled samples in the extractor cylinder is 2 cm, the duration of extraction period is shorter. At a temperature of 30 °C at sample top, 100 % of Collembola and Acari are obtained after 25 h from a sandy loam and after 50 (Collembola) to 90 (Acari) h from bog soil. Higher temperatures at sample top do not shorten the duration of extraction of Collembola irrespective of the soil type. On the contrary, the duration is considerably reduced for Acari from bog soil samples if the temperature at sample top is 40 °C. In general, the Macfadyen extractor gives better results compared to the flotation method. A sample thickness of 2 cm seems to be very convenient for the extraction of microarthropods from bog soil (Table 3).

Table 3. Numbers of Collembola and Acari obtained in the Macfadyen extractor (MF) *versus* Flotation (Fl)

°C	Th cm	Sandy loam						Bog soil					
		Collembola			Acari			Collembola			Acari		
		MF	Fl	REE	MF	Fl	REE	MF	Fl	REE	MF	Fl	REE
30 °C	2	18 1)	15 1)	120.0	158	176	89.8	522	393	132.8	220	155	141.9
35 °C	2	1766	1252	141.1	315	204	154.4	782	652	119.9	204*	70	342.9
40 °C	2	—	—	—	—	—	—	919*	205	448.3	136*	80	170.0
Total		**1784**	1297		**373**	380		**2223**	1250		**596**	305	
30 °C	4	161	109	147.7	246	176	139.8	355	393	90.3	171	155	110.3
35 °C	4	1926*	1252	153.8	500*	204	245.1	209	652	32.0	182*	70	260.0
40 °C	4	694	727	95.5	216	226	95.6	192	205	93.7	161	80	201.3
Total		**2781**	2088		**962**	606		756	**1262**		**514**	305	

1) Isotomidae only.

* Significantly higher values (Kruskal-Wallis-Test, $p = 0.05$).

Explanation of symbols: °C = temperature at sample top; Th = thickness of the sample in the extraction cylinder; REE = Macfadyen extraction efficiency in % of flotation efficiency.

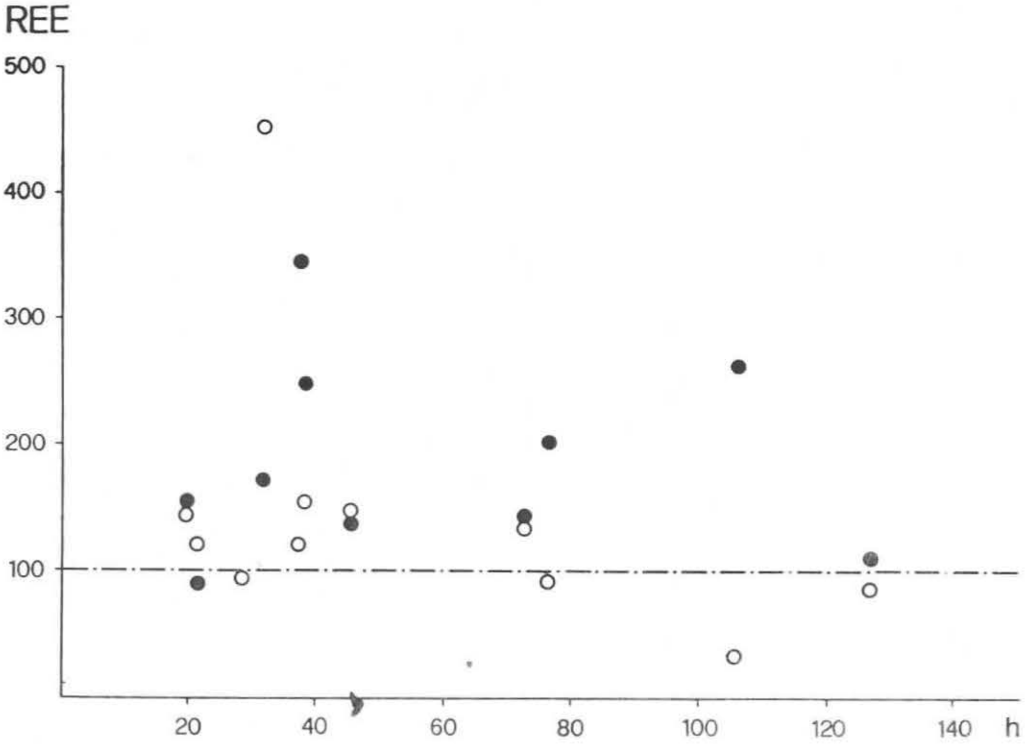

Fig. 3. Relative extraction efficiente (REE) for Collembola (○) and Acari (●) plotted against desiccation time (lowest desiccation limits = 10 % water content in bog soil and 2 % in sandy loam).

Table 4. Average handling time in min for sorting and counting all soil microarthropods of a sample by two different methods

Sample volumes	A Sorting by hand min	B Glycerol clean-up min	B in % of A
(133 cm³)	70.0	56.0	80.0
(266 cm³)	71.6	56.0	78.0
(362 cm³)	110.0	81.0	73.6
(724 cm³)	193.3	102.0	52.8

Table 5. Means of the square roots and t-values of the taxonomic groups to compare sorting by hand (36 samples) with clean-up by glycerol (40 samples)

Taxonomic category	Sorting by hand	Glycerol clean-up	t value
Total arthropods (per sample)	14.80	14.84	0.03
Collembola	6.97	6.84	0.27
Acari	12.51	12.60	0.10

3.4. The counting technique

Counting by hand of Macfadyen extracted organisms was compared with counting using the glycerol clean-up. The results are summarized in Tables 4 and 5, which show that there is a reduction of handling time after clean-up and that this increases with increasing sample size. Clean-up does not cause any loss in number of organisms (Table 5).

4. Discussion

As demonstrated by Vannier (1970) the main factor forcing animals to abandon a soil sample is soil water. It is therefore of importance that the desiccation process of a sample be adapted to the behaviour characteristics of the organisms being extracted. However, extraction conditions are for the most part established empirically (Macfadyen 1961; Murphy 1962; Edwards & Fletcher 1971; Lasebikan *et al.* 1978; Petersen 1978; Takeda 1979; Andren 1984), so that results obtained are difficult to compare even when the same equipment is used. To optimize extraction conditions an attempt was made to find out whether a relationship exists between the desiccation process of a sample and extraction efficiency. For this purpose the desiccation process of the samples was first estimated for the different soil types and at different extraction temperatures (Figs. 1 and 2). The estimated values were then plotted against the relative extraction efficiency (REE, Table 3), which is defined as the proportion of organisms extracted by the Macfadyen as compared with the number obtained by flotation (= 100%). It appears from Fig. 3 that greatest efficiency with the Macfadyen extractor is obtained when the desiccation process of the whole sample lasts between 40 and 60 h for Collembola as well as for mites. The advantages of this are a better use of the equipment and the possibility of storing soil samples for a short period of time without any consequence to their fauna composition (Edwards & Fletcher 1971). The methodology described here permits one to define with ease the optimum extraction conditions for a particular soil type or species, thus allowing each worker to adapt conditions to his needs.

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With a modified, newly constructed Macfadyen extractor highest numbers of Collembola and Acari are obtained when (a) samples are crumbled, and (b) sample temperature top is about 35 °C. These conditions apply to any type of soil, but the thickness of the sample in the extraction cylinder has to be adapted to the soil type. For a light mineral soil, like sandy loam, a thickness of 4 cm seems to be ideal for the extraction of both Collembola and Acari, whereas for bog soil the thickness should be reduced to 2 cm. At 35 °C maximum extraction was obtained within 40 to 60 h. A new method for determining the efficiency of dynamic extractors for soil microarthropods is proposed.

Key words: Macfadyen extractor, soil arthropods, extraction efficiency, temperature, Collembola, Acari.